

**U.S. Department of Energy (DOE)  
Bioenergy Technologies Office (BETO)  
2019 Project Peer Review**

**Bio-syngas to Fatty Alcohols (C6-14) as a  
Pathway to Fuels**

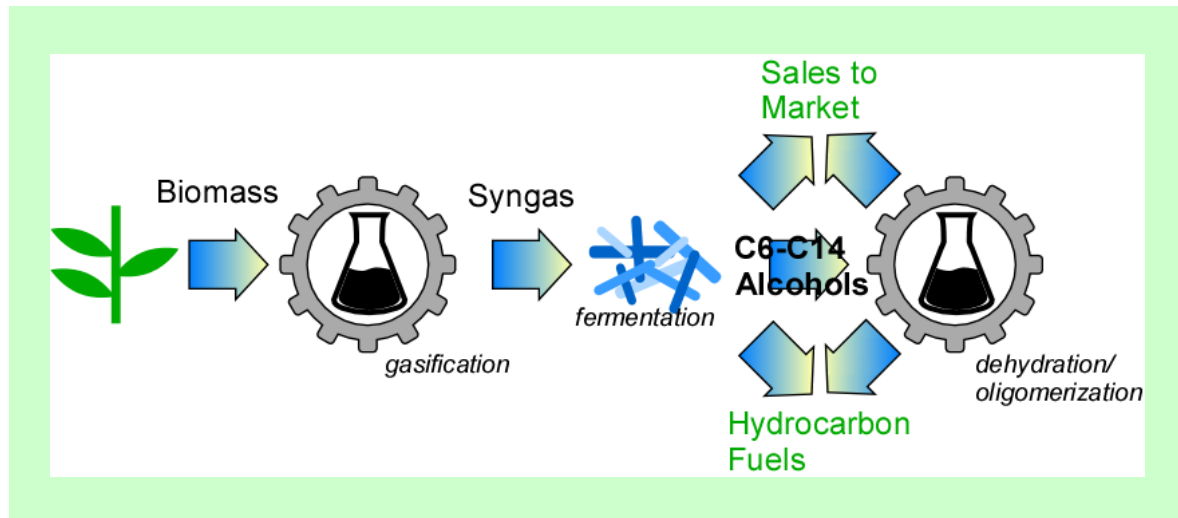
3/6/19

Biochemical Conversion

Principal Investigator: Devon C. Rosenfeld  
The Dow Chemical Company

# Project Goal

Develop a new bio-syngas fermentation process using engineered bacteria for production of intermediate (C6-C14) fatty alcohols leveraging robust chemical markets enabling scaling to biofuels production at < \$3/gge.



Aligned with Conversion R&D strategic goal of developing commercially viable technologies for converting biomass feedstocks via biological and chemical routes into energy dense, fungible, finished liquid transportation fuels and well as chemical intermediates.

# Quad Chart Overview



## Timeline

- Project start: 10/1/2016
- Project end: 5/31/2019
- 80% complete

## Budget

	FY 17 Costs Actual	FY 18 Costs Actual	FY 19 Costs Through Dec 31 <sup>st</sup>	Total Funding
<b>DOE Funded</b>	\$443,757	\$453,398	\$286,778	\$1,988,690
<b>Project Cost Share (Dow)</b>	\$128,872	\$157,742	\$32,415	\$419,071
<b>Project Cost Share (LanzaTech)</b>	\$104,770	\$83,391	\$110,792	\$580,642
<b>Project Cost Share (Northwestern )</b>	\$13,408	\$10,065	\$0	\$34,233

## Barriers Addressed

- Ct-D. Advanced Bioprocess Development
- Ct-H. Gas Fermentation Development

## Objective

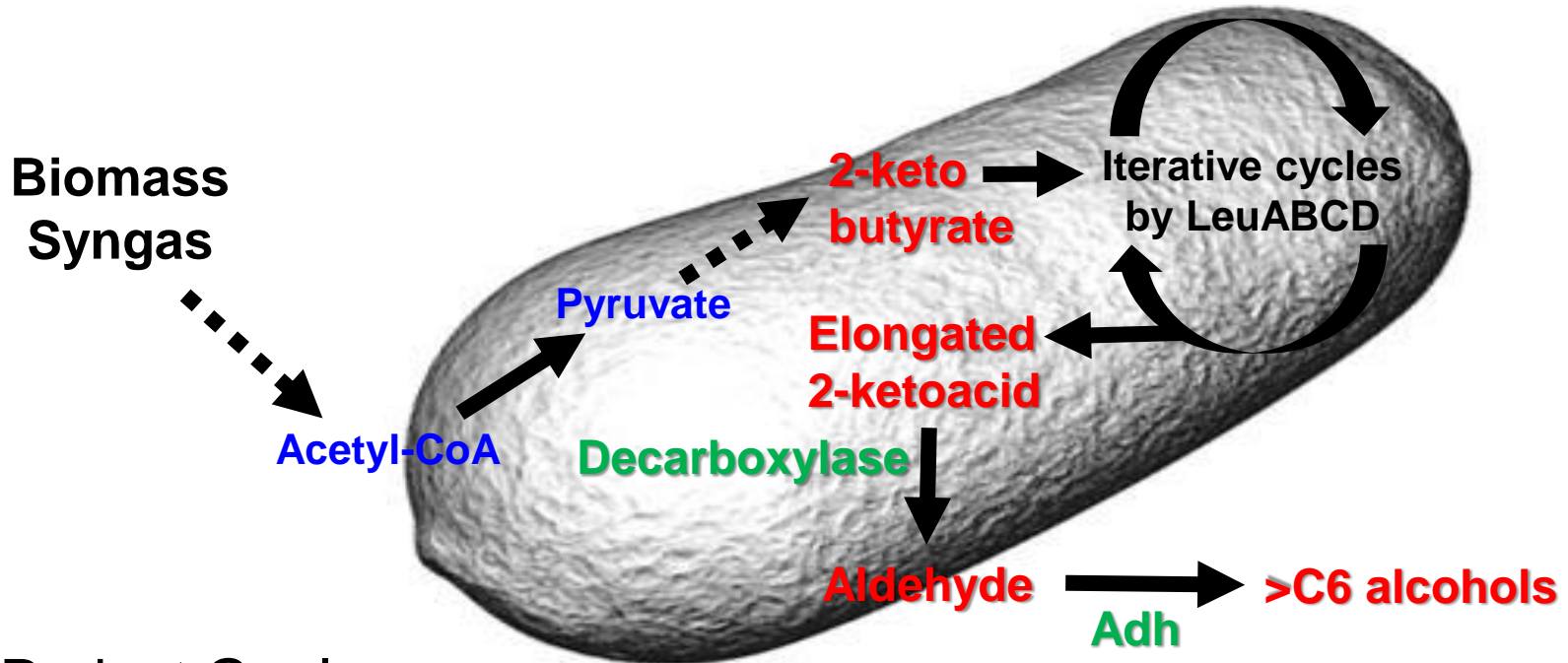
Develop a process for syngas fermentation to C6-C14 alcohols using engineered bacteria.

## End of Project Goal

C6-C14 alcohols from syngas demonstrated at a total titer, productivity and selectivity meeting TEA metrics to produce biofuels at price of < \$3/gge.

# Project Overview

- Dow and LanzaTech previously validated syngas conversion to fatty alcohols via the +1 pathway (LeuABCD) in *Clostridium*



- **Project Goals**

- Resolve pathway bottlenecks limiting yield
- Optimize strain to drive flux through the pathway
- Optimize fermentation to deliver titer, productivity and selectivity targets
- Develop a conceptual flowsheet for separating and purifying products

# Management Approach

- Dow is the project lead, coordinating participant activities.
- Teleconference every two weeks and annual in person team meetings to monitor work progress ensure timely milestone completion, and address any issues that may arise.
- Project teams organized according to alignment of core expertise and capabilities to delivery for specific project task.

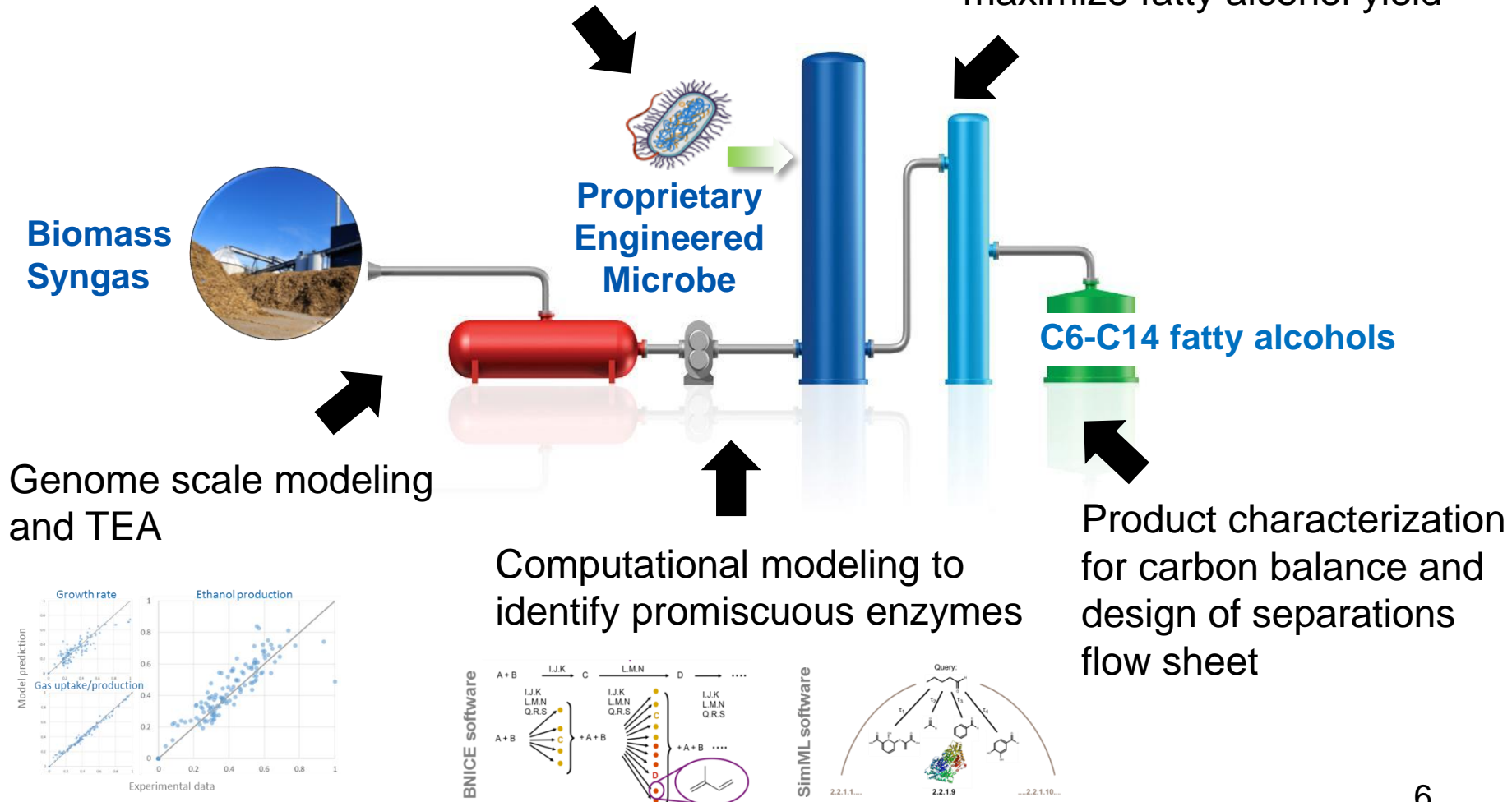
Project Lead	Tasks	Description
Dow	4 (BP2), 10	Intermediate and product characterization and project management
LanzaTech	1-3, 4 (BP3), 6-9	BETO validation, strain and fermentation optimization
Northwestern	5	Computational modeling for pathway development

- Successful task delivery through cross partner collaboration
- Go/No-Go milestone with fatty alcohol titer and productivity targets and updated techno-economic assessment (TEA) at the end of budget period 2 to assess our progress toward final targets.

# Technical Approach

Strain engineering to resolve previously identified challenges and eliminate unproductive pathways

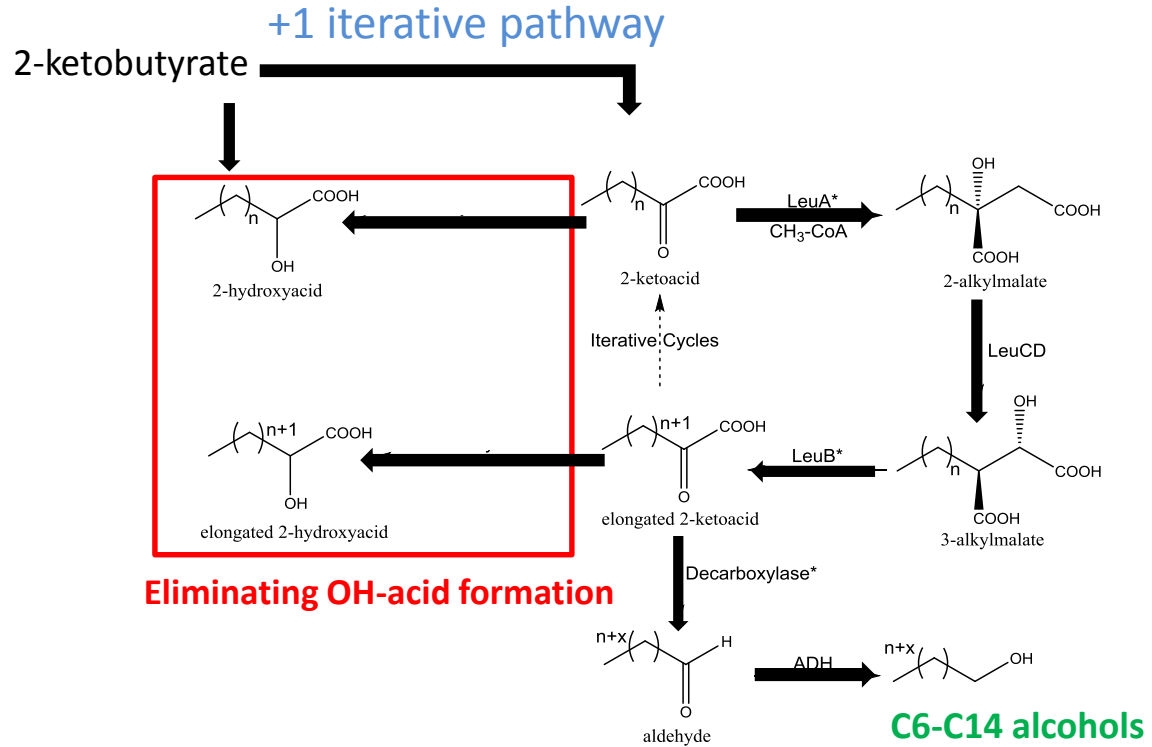
Fermentation optimization to maximize fatty alcohol yield



# Challenges to Project Success

## Technical Challenges

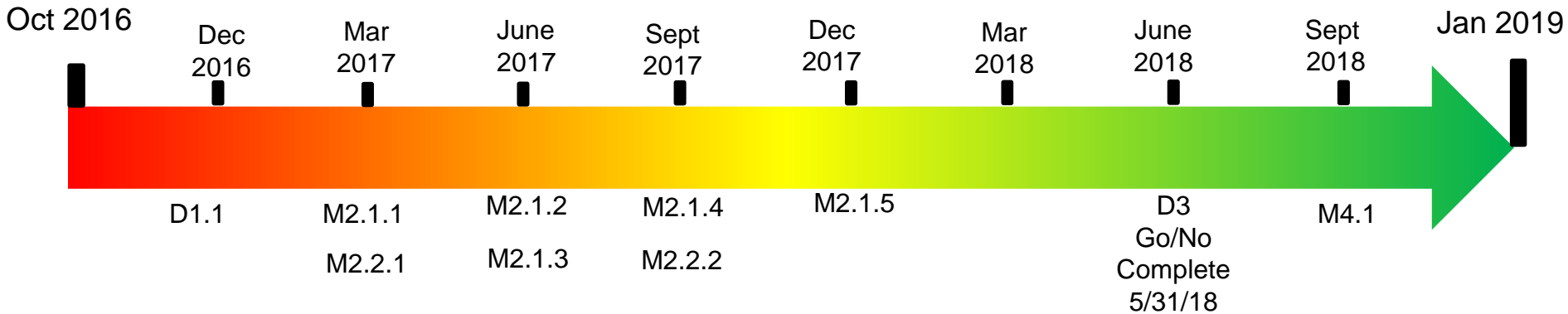
1. Pathway bottlenecks
2. Byproduct formation
3. Fermentation stability



## Commercial Challenges

1. Product separation and purification sufficient for commercial use
2. Sufficient co-product production to support scaling for fuels production

# Technical Accomplishments



- ✓ **BETO Baseline Validation (D1.1)**
- ✓ **Strain Engineering (M2.1-2.2)**
  - resolve bottlenecks and minimize byproduct formation
  - Computational modeling of key reactions and enzymes responsible
  - +1 pathway enzyme and *Clostridium* knock out strain screening
  - Lab scale fermentation with optimized strains
- ✓ **Product Identification (M4.1)**
- ✓ **D3: Go/No-Go Milestone:** C6-C14 alcohol from syngas demonstrated at 50% of final target total titer and 5% of final target productivity in 2L-CSTR bioreactor, GEM and TEA updated

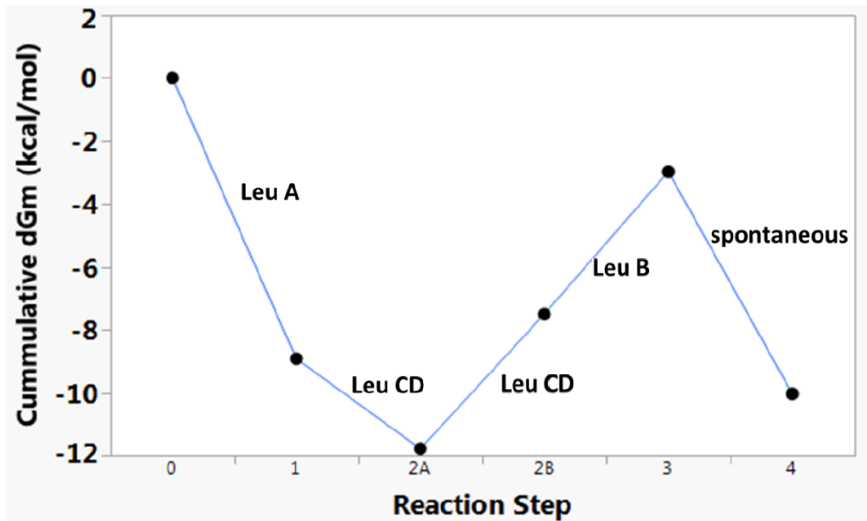


# Strain Engineering

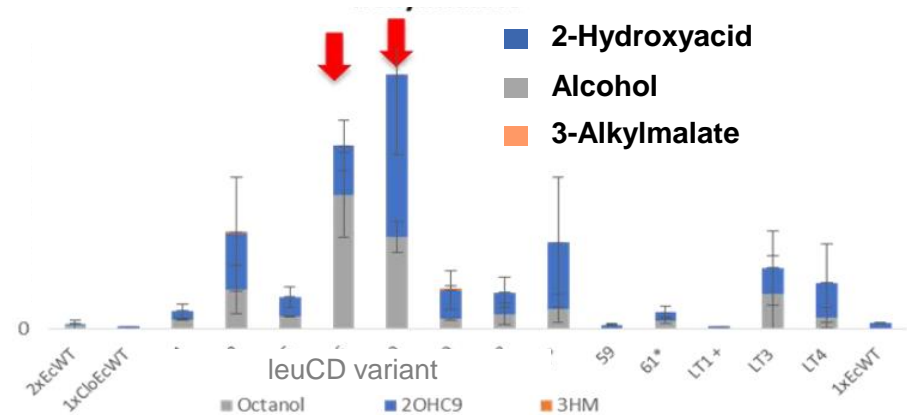
## Challenge 1: Resolve Pathway Bottlenecks

- ✓ M2.1.1: Identified >10 alternative pathways to LeuCD for conversion of C4-C14 2-alkylmalates to 3-alkylmalates via computational predictions
- ✓ M2.1.2: Built 17 strains with best performing LeuCD variants
- ✓ M2.1.3: Completed +1 pathway thermofeasibility assessment with SimZyme/SimML
- ✓ M2.1.5: Demonstrated up to 40-fold improvement in LeuCD activity *in vivo*

### +1 Pathway Thermodynamics



### LeuCD Variant Screening in *Clostridium* by Feeding 2-Alkylmalate

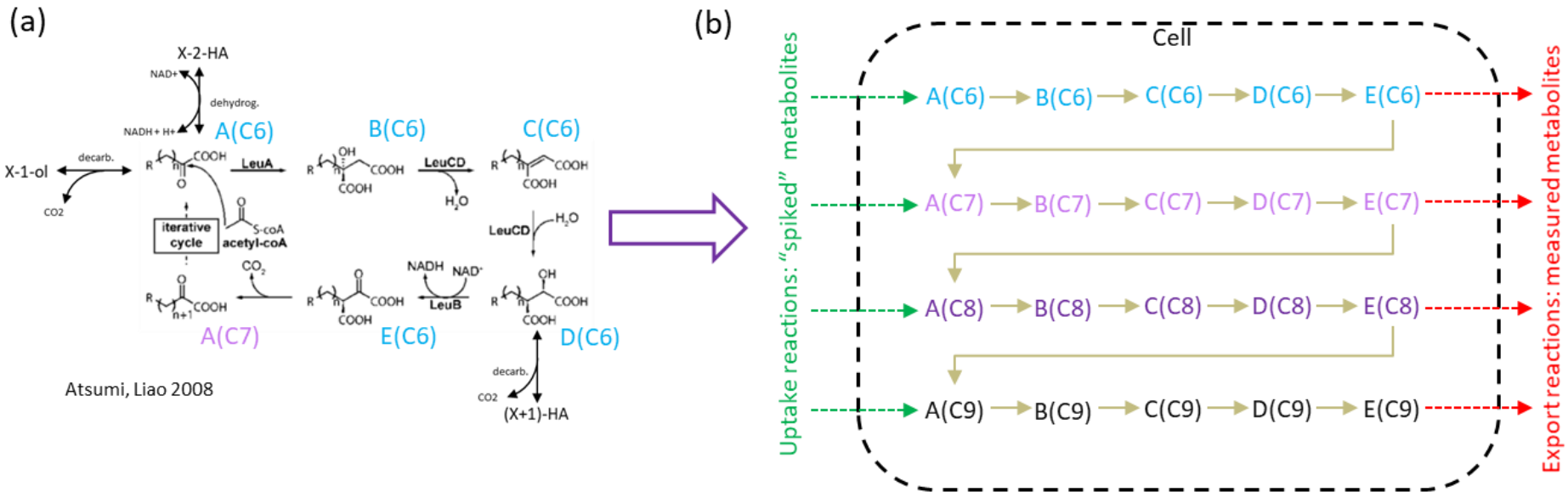


# Strain Engineering

## Challenge 1: Resolve Pathway Bottlenecks

✓ **M5.1.1**: Develop kinetic model to identify major factors limiting product flux and identify engineering strategy to mitigate root causes

### Ensemble Model of +1 Pathway

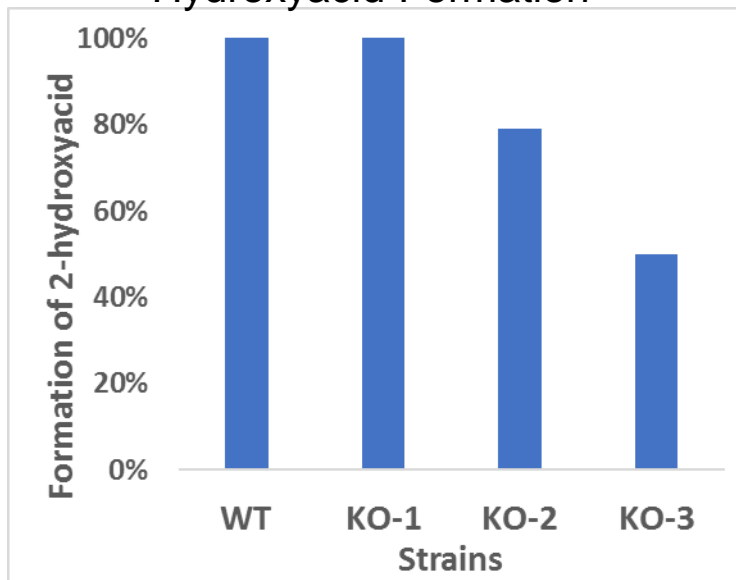


# Strain Engineering

## Challenge 2: Minimize Byproduct Formation

- ✓ M2.2.1: Predicted native *Clostridium* enzymes acting on +1 pathway via GEM
- ✓ M2.2.2: Constructed double and triple knock-out strains using GEM as guidance
- ✓ M2.2.2: Up to 50% reduction in 2-hydroxyacid formation demonstrated

KO Strain Reduction in 2-Hydroxyacid Formation



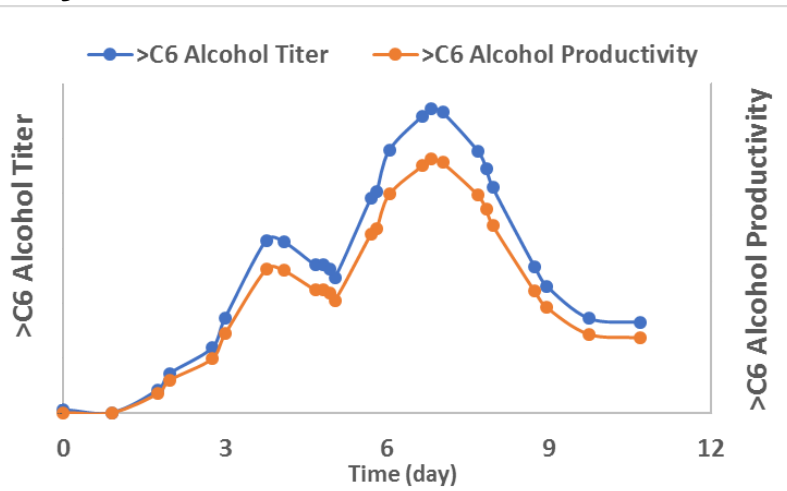
## Decarboxylase Variant *in vivo* Test

	Variant 1	Variant 2	Variant 3
>C6 Linear Alcohols	+	+	+
>C6 Aromatic Alcohols	++	++	+++
Branch-Chain Alcohols	+++	+++	-

# Fermentation Optimization

## Challenge 3: Fermentation Stability

July 2018

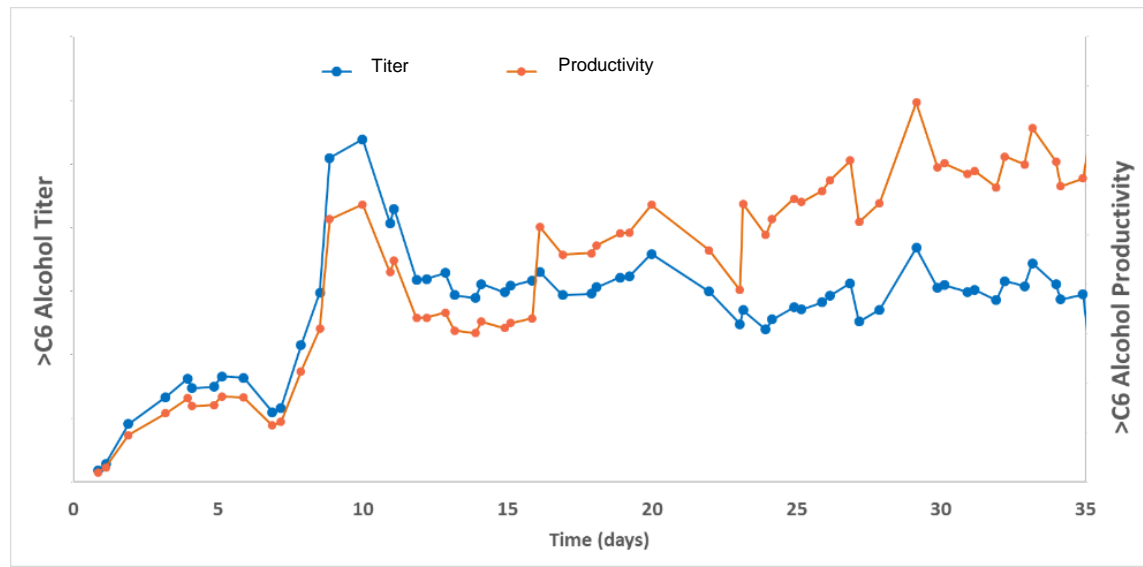


Continuous lab scale fermentation data

Fermentation conducted with synthetic gas mix mimicking corn stover

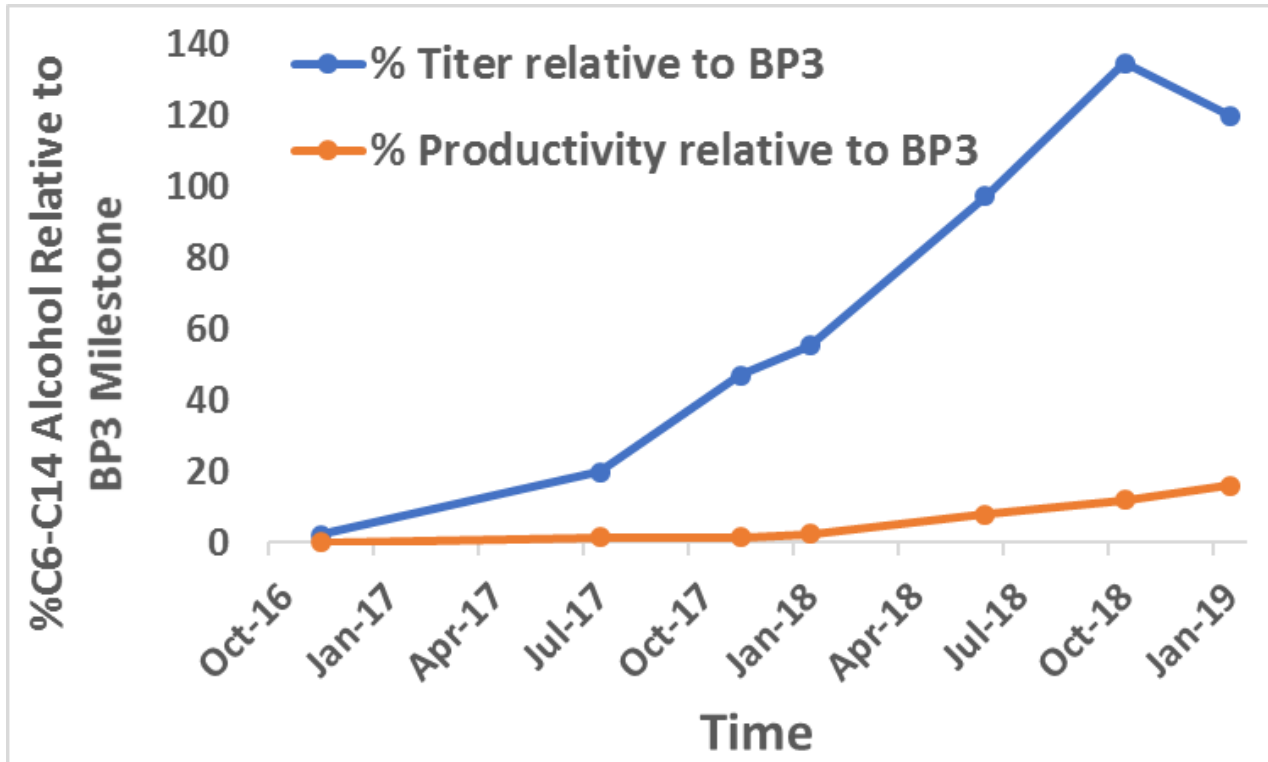
Stability improved through media and fermentation parameter optimization

October 2018



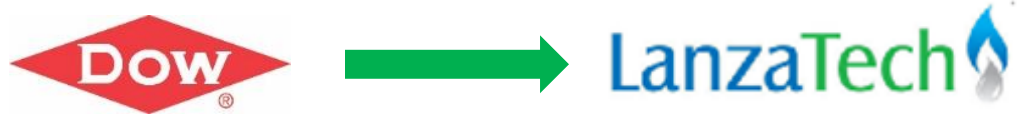
# Fermentation Optimization

- ✓ **D1.1**: Demonstrated C6-C14 alcohol production from syngas at 0.5% of target total titer in 2L CSTR bioreactor
- ✓ **D3 (Go/No-Go)**: C6-C14 alcohol from syngas demonstrated at 50% of final target total titer and 5% of final target productivity in 2L-CSTR bioreactor, GEM and TEA updated



# SOPO Updates

## *Partner Accountable for Milestone Completion*



M4.1.1: Identify all products

M4.2.2: Analytical method development to track products

M6.1: Develop product separation flowsheet

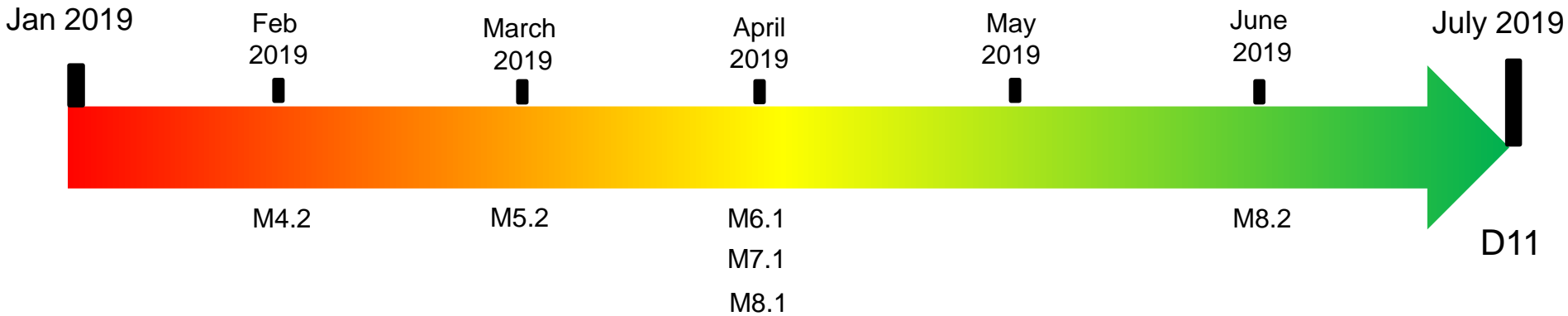
## *Redefined Milestone 5 Scope to Best Drive Project Success*



M5.1.1: Develop kinetic model to identify major factors limiting product flux and identify engineering strategy to mitigate root causes

M5.2.1: Model metabolic pathways to best drive improved flux

# Future Work



- Four areas of focus in 2019 leading up to project Go/No-Go
  - Utilize kinetic model results to guide strain optimization (M5)
  - Product separation flowsheet (M6)
  - +1 pathway enzyme selection and *Clostridium* knockout strain deployment (M7)
  - Fermentation optimization (M8)
- Deliver final project targets 7/31/19
  - C6-C14 alcohols from syngas demonstrated at a total titer, productivity and selectivity meeting TEA metrics to produce biofuels at price of < \$3/gge. Update GEM and TEA model.

# Relevance

Develop a new bio-syngas fermentation process using engineered bacteria for the production of intermediate (C6-C14) fatty alcohols robust chemical markets enabling scaling to biofuels production for sale at < \$3/gge

- Our project is aligned with BETO's vision and mission statements and strategic goals.
- Our technology will impact the industry and deliver BETO goals by:
  1. Creating a disruptive bioconversion technology leveraging robust chemical markets to traverse the "valley of death" of biofuels scaling
  2. Enabling feedstock versatility and decoupling raw materials from food crops.
  3. Displacing petroleum derived fuels and chemicals with domestically produced, cost competitive bio-renewables with improved infrastructure compatibility.
  4. Exceeding advanced biofuels GHG reduction target of >50% versus conventional
- Our technology has the versatility to potentially function as a front end upgrading process within an integrated biorefinery or to bolt on within conventional conversion infrastructure such as an FT refinery.



# Summary

**Goal:** Develop a new bio-syngas fermentation process using engineered bacteria for production of intermediate (C6-C14) fatty alcohols leveraging robust chemical markets enabling scaling to biofuels production at < \$3/gge.

- *Overview:* Rooted in prior research by Dow and LanzaTech validating syngas fermentation to fatty alcohols via the +1 pathway with yield limiting challenges
- *Approach:* Deploy strengths of three partners to resolve challenges, maximize fatty alcohol titer and devise purification scheme
- *Technical Accomplishments:* Completed all BP2 milestones including Go/No-Go milestone.
- *Relevance:* Aligned with BETO's long term vision and deliver MYPP goals
- *Future Work:*
  - BP3: maximize fatty alcohol titer through strain and fermentation optimization, full product characterization for designing separation and purification flow-sheet

# Acknowledgements



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Loan Tran  
Sarah Ye

Linda Broadbelt  
Jennifer Green  
Jake Martin  
James Metz  
Joseph Ni  
Jonathan Strutz  
Keith Tyo

# Additional Slides

**(Not a template slide – for information purposes only)**

- *The following slides are to be included in your submission for Peer Evaluation purposes, but will **not** be part of your oral presentation –*
- *You may refer to them during the Q&A period if they are helpful to you in explaining certain points.*

# Responses to Previous Reviewers' Comments

We appreciate the reviewers' feedback. We have provided responses to the most important comments on this and the next slide.

- 1) Multiple comments were provided regarding the sensitivity of the system to syngas composition. LanzaTech's proprietary strains of *Clostridium* function on a wide range of syngas compositions. Therefore studying *Clostridium's* response to changing syngas composition is out of scope for this work.
- 2) Two reviewers asked for information on the technical challenges. The foremost technical challenges for our project are included in our 2019 Project Peer Review presentation.

Note: This slide is for the use of the Peer Reviewers only – it is not to be presented as part of your oral presentation. These Additional Slides will be included in the copy of your presentation that will be made available to the Reviewers.

# Responses to Previous Reviewers' Comments

- 3) Two reviewers commented on our choice of pathway and each suggested alternatives. We have considered the *Clostridial* chain elongation and reverse beta oxidation routes. However, these routes are limited to production of straight and even number chain length alcohols. Additionally to our knowledge, hexanol is the longest chain alcohol shown to be produced through *Clostridial* chain elongation. The +1 pathway has the advantage that in addition to branched chain alcohols odd chain alcohols can also be accessed. Expression of *E. coli* enzymes is not considered a challenge, as LanzaTech has developed a sophisticated codon usage algorithm and routinely expressing *E. coli* enzymes without issues (to date LanzaTech has expressed over 100 different *E. coli* enzymes).

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# Highlights from Go/No-Go Review (May 2018)

- Delivered all BP2 milestones
- Generated 136 new strains
- Delivered BP2 Go/No-Go milestone (D3)
  - 180% titer
  - 190% productivity
- DOE Intermediate Validation Team Recommendations
  - Determine and then focus on biggest inhibitor to achieving sustained alcohol production
  - Continue focus on strain development

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# Publications, Patents, Presentations, Awards, and Commercialization

- Publications, patents, awards, and presentations
  - One invention disclosure filed and patent application in preparation
  - No publications or presentations
- Describe the status of any technology transfer or commercialization efforts
  - No technology transfer or commercialization efforts to report

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